Monitoring microbial water quality in Nordhavn

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February 2015

VAND I BYER
KLIMATILPASNING OG INNOVATION

DTU Environment
Department of Environmental Engineering

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Preface

Nordhavn is an ambitious project that is meant to demonstrate Copenhagen's future sustainable city. Nordhavn will establish 40,000 residents and 40,000 jobs in a sustainable city that showcases sustainable solutions in energy, climate, and water.
The "Nordhavn - en bydel i vandbalance" project, which is an innovation project (IP12) in the larger "Vand I Byer" project, was established to demonstrate how new technology can be used to minimize the impact on water resources. This project had three sub activities. This report contains the results of one of the sub activities, which focused on the extent to which water quality can be monitored with innovative on-line sensors that can be used to detect contamination and give an alarm if microbial water quality is possibly compromised.

To examine this, we did a microbial investigation at two monitoring stations in Norhavn. We frequently measured many traditional parameters used to access *microbial* water quality including heterotrophic plate counts using a yeast extract medium at 22 and 37 °C and a lower nutrient R2A medium, as well as ATP and total direct counts. This was done over an extended period of time, and compared them with the parameters being monitored at the stations to try and establish a correlation. The monitoring stations were established to monitor water quality using new and traditional water quality parameters including temperature, dissolved oxygen, turbidity, nitrate, TOC, and DOC, as well as monitoring UV absorbance at 245 and 400 nm. A newly developed sensor, the Zebra, was also used to quantify microbial and particle counts on a nearly continuous basis. The sampling campaigns were designed to determine the microbial water quality over different times, including several days of sampling during peak use hours, an overnight campaign to determine microbial water quality during non-peak use hours, and an extended campaign that sampled at one time over the period of a month.

DTU Environment, February 2015

Content

1. Background

Two monitoring stations were established at different locations in Nordhavn. One monitoring station was set up on Sundkrogsgade, and the other on Nordsøvej (Figure 1). The station at Sundkrogsgade was set up inside a container with a line that directly connects to the distribution system. The Nordsøvej station was set up inside a government building. The Sundkrogsgade station was situated in an area with higher water demand and which was closer to the main water distribution line supplying Nordhavn. The second monitoring station was established at the end of Nordhavn, which was farther out in the distribution system than the station at Sundkrogsgade and had considerably less water demand. Therefore a much longer residence time and water age is expected at the Nordsøvej station compared with the Sundkrogsgade station. This increased residence time and water age can increase microbes in the water and lead to deteriorating microbial water quality.

Figure 1: Map of the locations of the monitoring panels in Nordhavn

1.1 Monitoring panels

The monitoring panels established at both sites in Nordhavn were identical and consisted of continuous monitoring of several parameters, which can be seen in Figure 2. Many of the monitored parameters are well established and regulated to ensure the quality of drinking water. Currently though, there is little information to relate the microbial water quality from these other established parameters. One of the main goals of this project was to investigate if the easily monitored, and rapidly determined parameters provided by the monitoring panels, could reflect the microbial water quality as measured by the traditional and more time consuming and non-automated methods normally used for determining the microbial quality of drinking water.

2. Materials and methods

Several parameters were analysed to evaluate the microbial water quality at the two monitoring stations. The different parameters analysed, and the corresponding methods, are described later in this section. All samples were collected at the same time at each station and several different sampling campaigns were done to analyse the microbial water quality over different scenarios.

2.1 Sampling campaign

The sampling campaign was designed to determine two main objectives. The first was to compare the monitoring panel data at the two stations and to determine if there was a possible relationship between the microbial water quality and the sensor panels. This was done to determine if the online sensors could detect a change in the microbial water quality, and if so, could this be used to determine microbial water quality. The second objective was to determine if there was a change in the microbial water quality as a function of time. This objective was accomplished by analysing changes in microbial water quality:

- Over the course of peak consumption hours (9:00 am to 3 pm) for 6 days staggered over two weeks. These peak consumption hours also corresponded to shifts in the source water.
- At one specific time over a period of several weeks (10:00 am).
- During an extended period of low use (3:00 pm- 10:00 am).

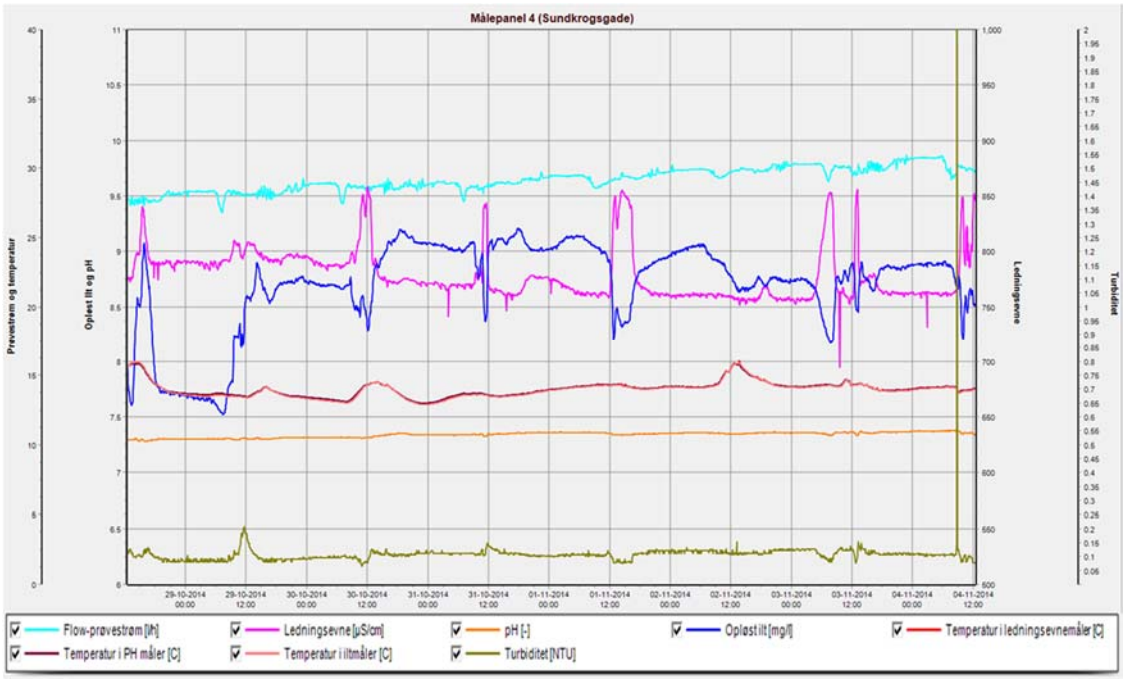


Figure 2: Sundkrogsgade sensor results over a period of 7 days (October 28-Nov 4, 2014) prior to the sampling campaign.

Before the sampling campaign began, it was established from the monitoring panels that there was a shift in the composition of the water at the Sundkrogsgade station that generally occurred around noon (Figure 2). This shift is not a normal occurrence, but during the sampling campaign Slangerup waterworks was not in

operation. It is thought that because of this, there could be a shift in the waterworks supplying Nordhavn during different parts of the day. This shift in water sources can be seen in the spikes in conductivity (EC) and dissolved oxygen (DO) in Figure 2. This provided a unique opportunity to examine the changes in microbial water quality caused by shifting water sources.

2.2 Sample collection and preservation

All samples collected for microbial water quality were analysed for heterotrophic plate counts (HPC) using yeast extract agar or R2A agar. To distinguish from the two different HPC agar results, the figures refer to the plate count analysis using yeast extract as HPC and as the plate count analysis using R2A agar as R2A. The Samples for the total direct count (TDC) were analysed for every other sample during the peak consumption hours sampling, at every 10:00 am sample, and for every fourth sample during the extended low use hours sampling campaign. Samples were collected from November 21st to December 17th, 2014. A total of 65 separate sampling times were analysed at both stations. HPC results were performed in duplicate at no dilution and at 1 dilution. HPC using yeast extract was examined at 2 temperatures and HPC using R2A was examined at 1 temperature, for a total of 776 separate plate counts. An additional 225 analyses were done to examine the TDC and ATP.

Following sampling, all samples were immediately put on ice until transported back to the lab immediately following a sampling event. Samples were then immediately plated for the R2A analysis, or delivered to ALS Laboratories, who conducted the HPC using yeast extract. Samples for TDC were also immediately preserved with buffered formaldehyde (2% of sample volume) and were stored at 4 °C until analysis (between 1-7 days). ATP samples were immediately stored at -80 °C until analysis.

Samples for HPC using yeast extract were collected in sealed sterile plastic bottles provided by ALS laboratories. ATP samples were collected in duplicate in sterile 6 mL plastic scantilation tubes. Samples for the TDC and HPC using R2A agar were collected in 100 mL glass bottles that were first acid washed and baked at 550 C° for several hours.

2.3 Heterotrophic plate counts (HPC) using yeast extract at 22 °C

Analysis for HPC using yeast extract at 22 °C was performed by ALS laboratories according to ISO 6222. Samples were performed in duplicate in undiluted water and at a dilution of 10 times. Samples were plated and incubated at 22 ± 2 °C for 68 ± 4 hours and are reported as HPC at 22 °C.

2.4 Heterotrophic plate counts (HPC) using yeast extract at 37 °C

ALS laboratories also did the analysis for HPC using yeast extract at 37 °C according to ISO 6222. Samples were performed in duplicate in undiluted water and at a dilution of 10 times. Samples were plated and incubated at 36 ±2 °C for 44 ±4 hours and are reported as HPC at 37 °C.

2.5 Heterotrophic plate counts (HPC) using R2A agar

HPC were also analysed using R2A agar as a medium. R2A is a low nutrient agar and could be a more useful growth media for nutrient poor environments such as drinking water. This method has shown to be more sensitive than nutrient rich plate count methods, such as those using yeast extract, in nutrient poor environments. Similar to the HPC using yeast extract, R2A analysis were done in duplicate for undiluted samples and at a dilution of 10 times. Samples were incubated for 14 days at 22 °C and counted at 7 and 14 days. The 14 day results were used because they show the same trends as the 7 day results with slightly higher counts as illustrated in Figure 3.

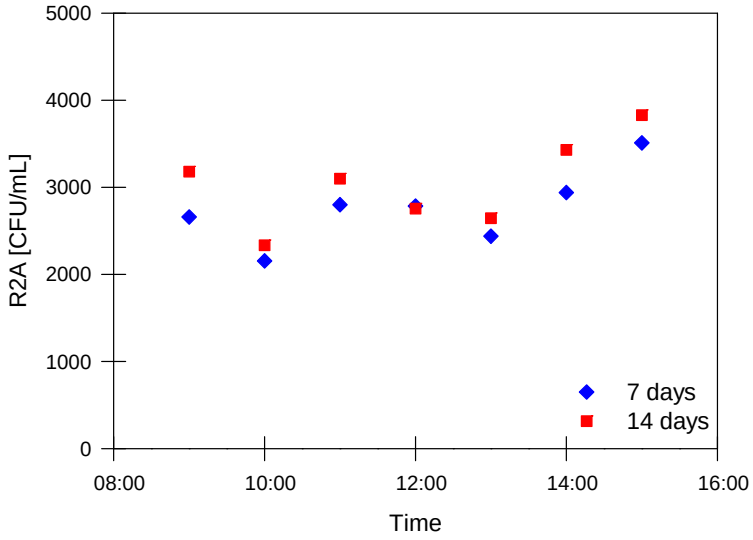


Figure 3: R2A results for Nordsøvej on November 24th, incubated for 7 and 14 days as a function of the time the sample was collected.

2.6 Total Direct Count (TDC)

The TDC was determined using fluorescence microscopy with the fluorescent dye DAPI (4',6-diamidino-2-phenylindole), which strongly binds to specific regions in DNA. The preserved samples were filtered through a 0.22 µm polycarbonate filter that was wetted with a 2% Tween-80 solution. To prolong fluorescence, 0.5 mL of a DABCO solution was added. Cells were enumerated using fluorescence microscopy where 10 to 20 grids different grids were counted. The coefficient of variation should not exceed 25% for reliability of the method.

2.7 ATP

Total ATP for all sampling times was analysed using a luminometer following extraction of microbial ATP using an extraction reagent (cell lysis) followed by a luciferin/luciferase agent. Extreme caution was taken to ensure samples were not contaminated including using laboratory equipment and pipette tips that were ATP free.

3. Results

3.1 Monitoring panels

3.1.1 Comparing values at Sundkrogsgade and Nordsøvej

Figure 4 shows the results from one of the monitoring panels at Sundkrogsgade and Nordsøvej. Although most parameters are similar to each other across the sampling period, there are a few noticeable differences and trends. Both EC and DO are approximately the same at both stations, but there is a much larger fluctuation in these parameters at the Sundkrogsgade station. This increased variability is likely due to the changing source water during this period, which is coupled with the greater water demand at the Sundkrogsgade station. The other notable difference is that the temperature at Nordsøvej is higher than at Sundkrogsgade and has much more variability throughout the day. The water temperature at Nordsøvej drops approximately 1.5 to 2 °C during the day, when the building is being used. This increased temperature and variability is

likely related to the residence time and water usage in the building where the Nordsøvej monitoring panel was set up.

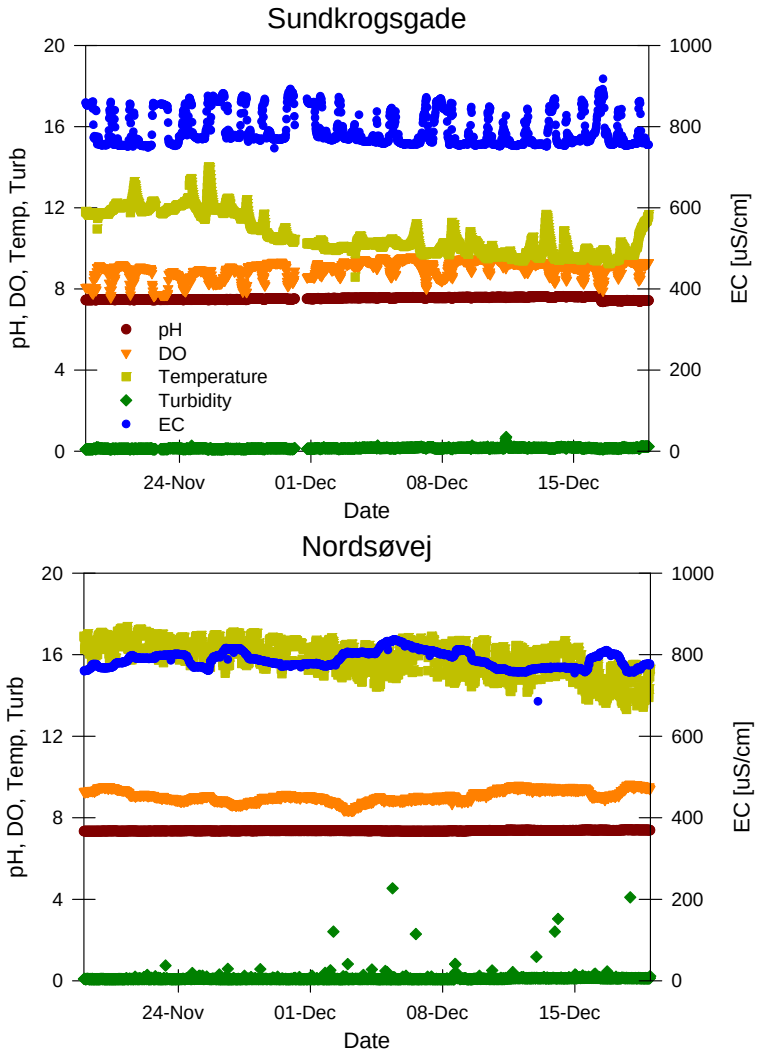
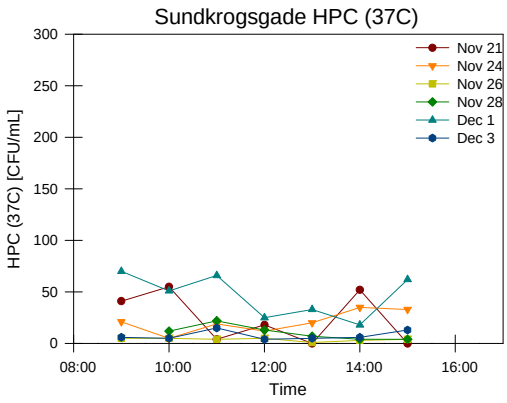


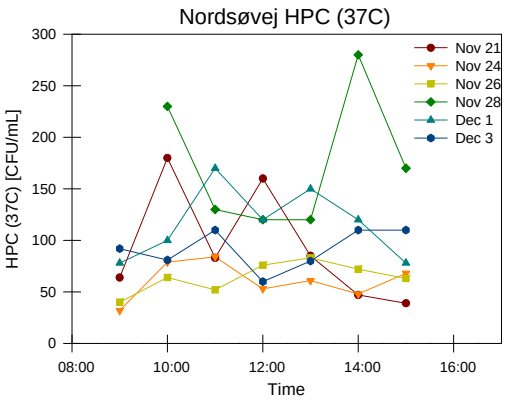
Figure 4: pH, DO (mg/L), Temperature (°C), Turbidity (NTU), and EC (µS/cm) results for the Sundkrogsgade and Nordsøvej monitoring panels over the period of the sampling campaign.

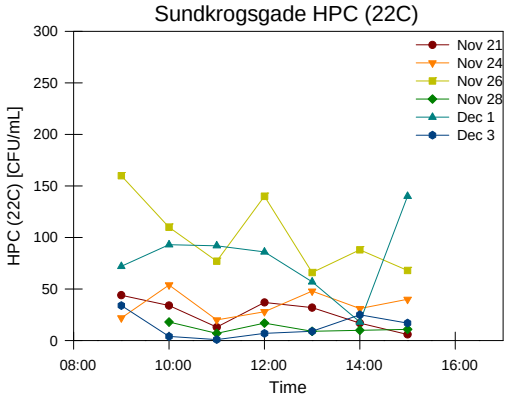
3.2 Microbial water quality

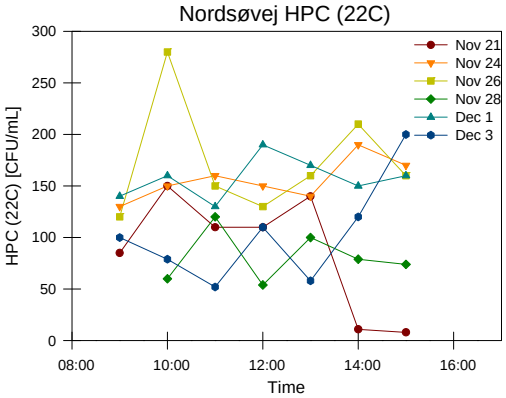
3.2.1 Microbial water quality during peak hours

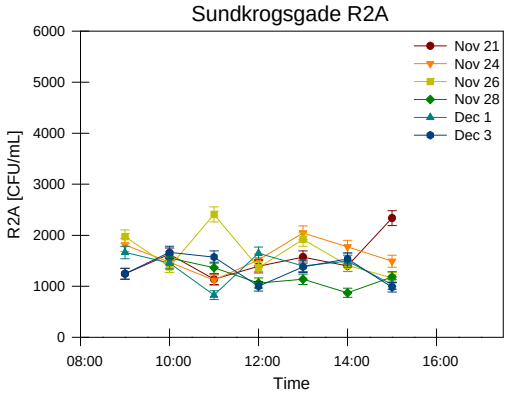
Earlier observations of an almost daily shift in the composition of the water led to a sampling campaign to analyse the microbial water quality over this period. This period also corresponds to what should be the peak water consumption rates. Samples were collected from 9:00 to 15:00 for six different sampling days, which began on Friday, November 21st. Samples were then collected every Monday, Wednesday, and Friday until the 3rd of December. Samples collected on the 28th of November were started at 10:00 instead of 9:00. All microbial water quality results for this sampling campaign are shown in Figure 5 and Tables 1 and 2 show all microbial water quality results for the entire project.

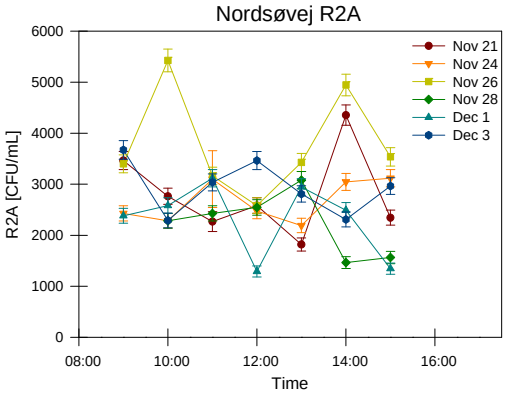

Sundkrogsgade HPC (37C)
HPC (37C) [CFU/mL]
Time
Nov 21
Nov 24
Nov 26
Nov 28
Dec 1
Dec 3

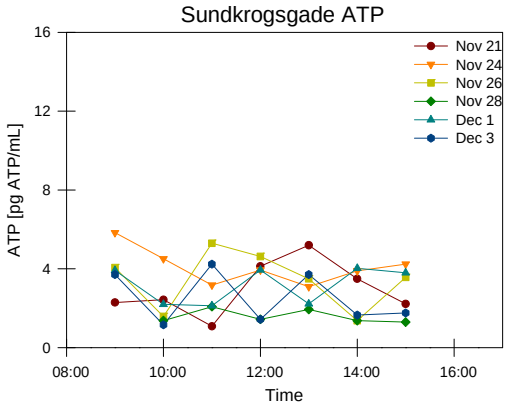

Nordsøvej HPC (37C)
HPC (37C) [CFU/mL]
Time
Nov 21
Nov 24
Nov 26
Nov 28
Dec 1
Dec 3

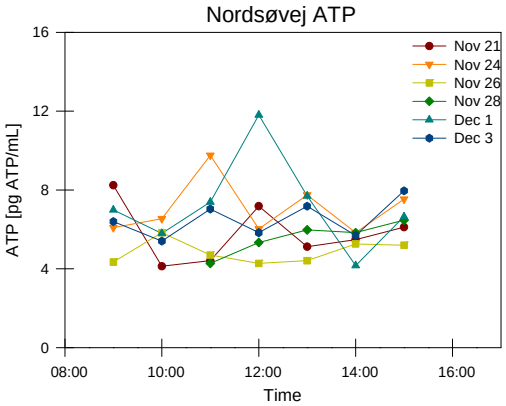

Sundkrogsgade HPC (22C)
HPC (22C) [CFU/mL]
Time
Nov 21
Nov 24
Nov 26
Nov 28
Dec 1
Dec 3


Nordsøvej HPC (22C)
HPC (22C) [CFU/mL]
Time
Nov 21
Nov 24
Nov 26
Nov 28
Dec 1
Dec 3


Sundkrogsgade R2A
R2A [CFU/mL]
Time
Nov 21
Nov 24
Nov 26
Nov 28
Dec 1
Dec 3


Nordsøvej R2A
R2A [CFU/mL]
Time
Nov 21
Nov 24
Nov 26
Nov 28
Dec 1
Dec 3


Sundkrogsgade ATP
ATP [pg ATP/mL]
Time
Nov 21
Nov 24
Nov 26
Nov 28
Dec 1
Dec 3


Nordsøvej ATP
ATP [pg ATP/mL]
Time
Nov 21
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Nov 28
Dec 1
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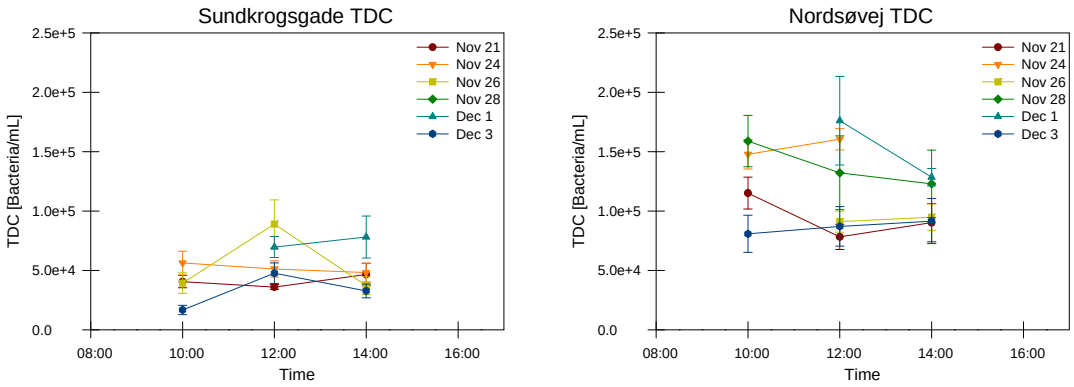


Figure 5: HPC using yeast extract at 22 °C, 37 °C, HPC using R2A, ATP, and TDC values for Sundkrogsgade and Nordsøvej monitoring stations over the period of peak water use hours (9:00-15:00) on six different sampling days.

Although a shift in the microbial water quality might be expected during the peak use hours, this was not observed. Figure 6 shows the average hourly values of all the microbial water quality parameters analysed over the 6 sampling days. It should be noted that the TDC values exceeding a COV of 25% were removed. This was done to ensure the integrity of the results. The results for 4 out of 18 samples at each station were removed, during the peak hours sampling campaign, because the COV was greater than 25%. All TDC values with their standard deviation and COV are listed in Tables 1 and 2 in the appendix. The average values shown in Figure 6, illustrate that the microbial water quality is relatively stable at both stations. This is despite the increased demand and the shift in water composition seen from the monitoring panel values at the Sundkrogsgade station (Figure 4).

A few key observations in microbial water quality were observed between the stations though. At the Nordsøvej station, all of the measured microbial water quality parameters were greater than at Sundkrogsgade and had larger variations. Over this sampling campaign, the R2A, HPC (22 °C), ATP, and TDC values were, on average, approximately 2-3 times larger at the Nordsøvej station. This could be expected though as the Nordsøvej station had a longer residency time and water age than Sundkrogsgade station. This might also be expected because of the 4-7 °C increase in temperature at the Nordsøvej station. This increased temperature and time in the distribution system can increase microbial growth. One useful parameter to know would be the residence time in the distribution system, especially the difference in residence time between the two monitoring stations, and the temperature of the water coming into the building. This information could be coupled with the current microbial water quality data to analyse the effect of residence time on the growth of microbes in the distribution system. It might also be useful to establish the Nordsøvej station with a direct link to the water coming into the building, as the increase in temperature and residence time in the building could have contributed to some of the observed increase in microbial growth.

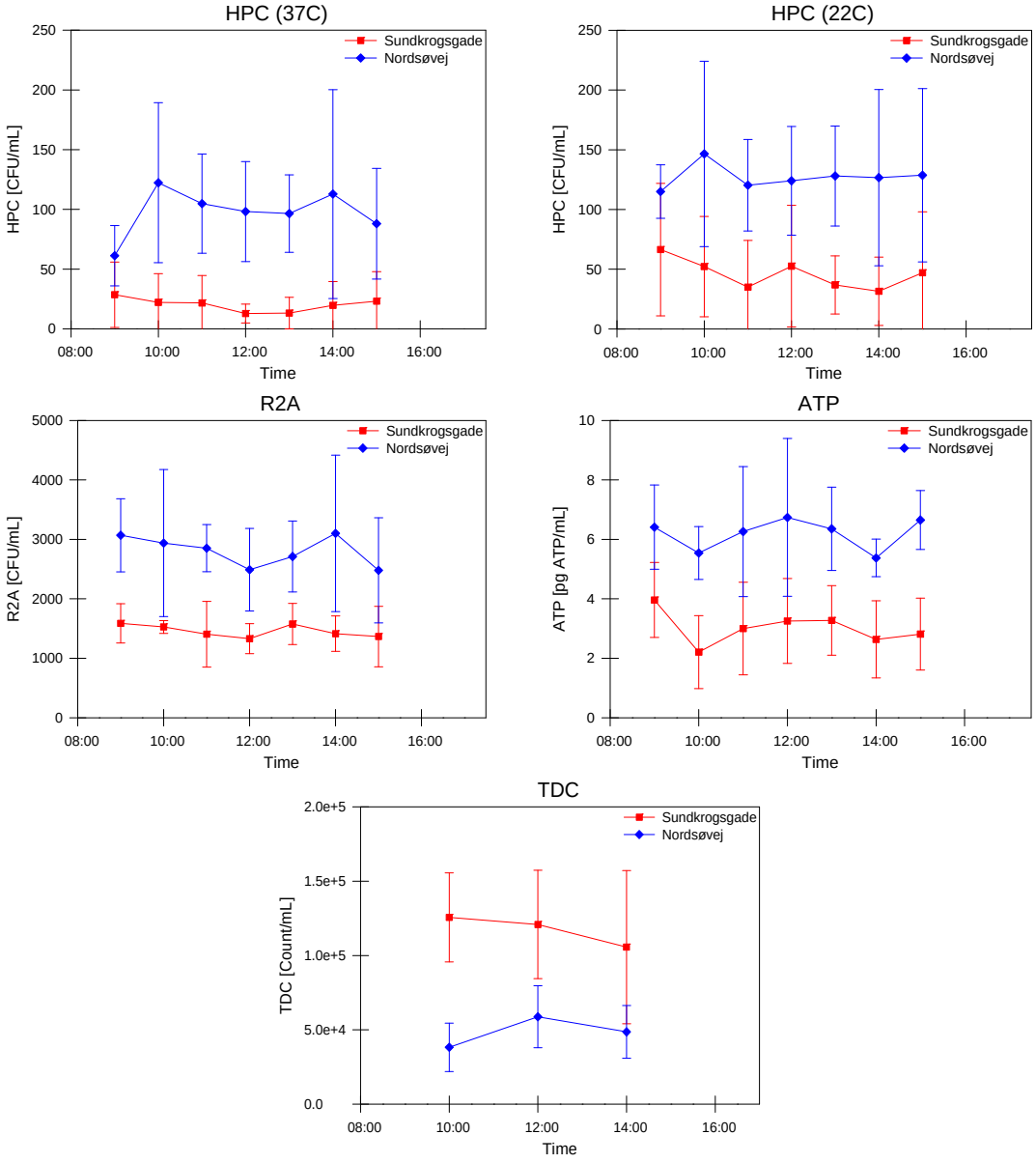


Figure 6: Average hourly values during peak consumption at Nordsøvej and Sundkrogsgade for HPC (37 °C), HPC (22 °C), R2A, ATP, and TDC. Values are average of 6 sampling days with error bars showing ± 1 std dev.

3.2.2 Sampling during non-peak water usage hours (15:00-10:00)

Samples were collected during an overnight sampling campaign starting at 15:00 on December 9th and continuing until 10:00 on December 10th. This was done to assess the microbial water quality over a period of decreased demand. In general the water quality parameters are similar to what was observed during the daytime sampling campaigns with Nordsøvej having higher numbers and greater variation (Figure 7). The two exceptions though were the first samples collected from the Sundkrogsgade station at 15:00 on December 9th, and the HPC at 22 °C results from the Sundkrogsgade station (Figure 7). The Sundkrogsgade samples collected at 15:00 show substantially increased counts for HPC at 22 and 37 °C, and the R2A samples. The R2A value of 3.8×10^3 CFU/mL was almost twice as high as the next highest value for the entire sampling campaign. The HPC at 22 °C was 895 CFU/mL, which was more than 5 times higher than any values on all other sampling days.

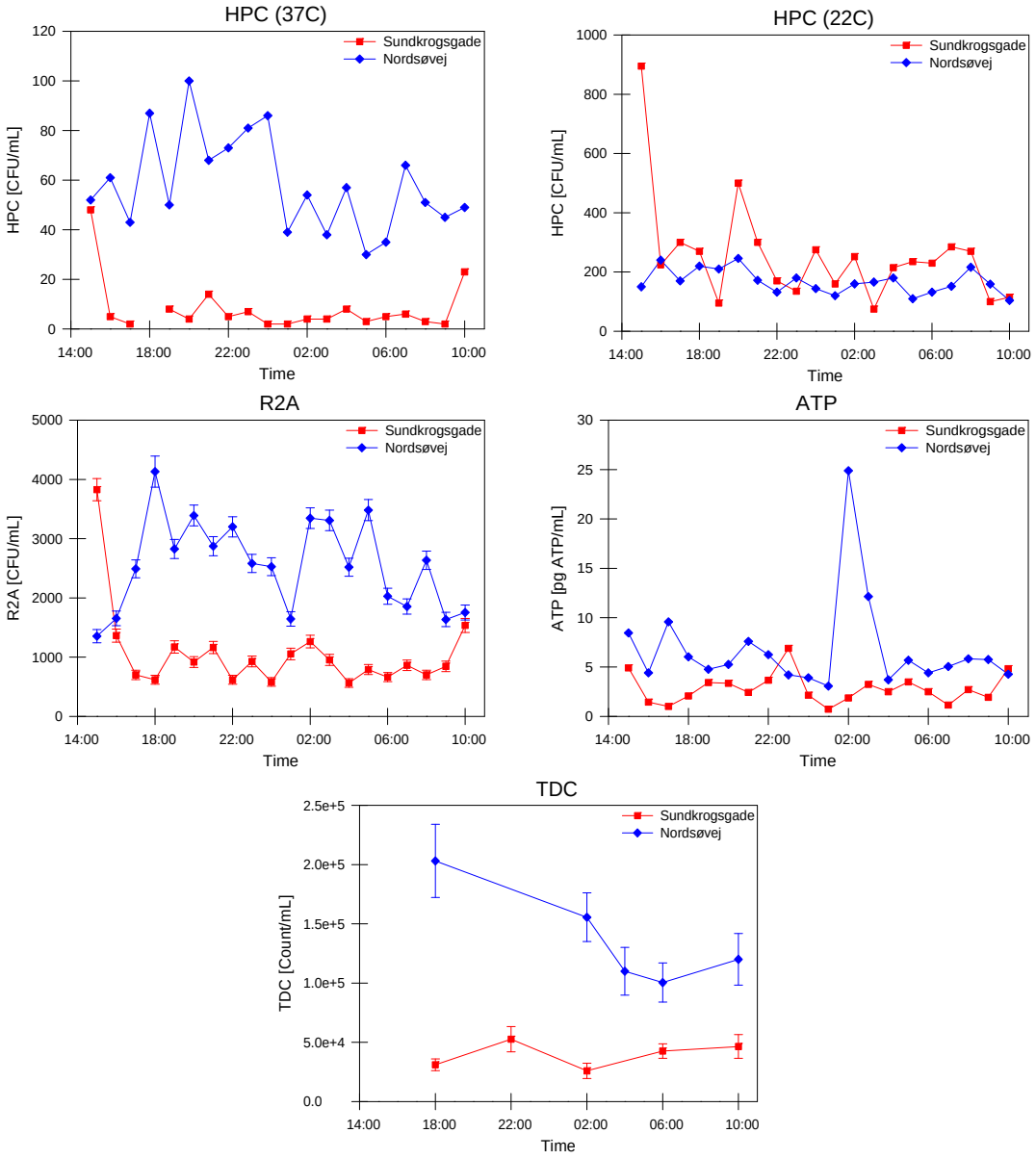


Figure 7: Sampling campaign results during non-peak hour water demand (15:00 Dec. 9th- 10:00 Dec. 10th) at Nordsøvej and Sundkrogsgade for HPC (37 °C), HPC (22 °C), R2A, ATP, and TDC.

In general, the Sundkrogsgade HPC values at 22 °C did not follow the general trends of having lower CFU/mL and less variation than Nordsøvej. The highest count observed during other sampling events was 160 CFU/mL. During the overnight campaign, a vast majority of the HPC at 22 °C samples measured higher than this. Also, the average R2A, ATP, and TDC results were approximately 2-3 times higher at Nordsøvej, similar to what was observed during the peak hours sampling campaign. The average HPC at 22 °C was 0.7 times lower at Nordsøvej, which does not fit the pattern observed for any of the other sampling events. This suggests that there could have been a systematic error in the sampling, preservation, or analysis of the HPC at 22 °C at Sundkrogsgade for this event. Contact with ALS laboratories showed that this was checked by their lab and therefore it is likely that these results were due to how the HPC at 22 °C samples were pre-served at Sundkrogsgade during this sampling event.

3.2.3 Microbial water quality at 10:00

In order to establish a longer term measuring campaign, samples were also collected at 10:00 at both stations for several other days. These samples, combined with the 10:00 peak hour samples and the 10:00 sample collected during the extended overnight campaign, were designed to examine the microbial water quality over an extended period. The samples collected at 10:00 showed some variation over the sampling period for the HPC using yeast extract at 22 °C and 37 °C. Despite the variation, the general trends of higher counts and greater variation at the Nordsøvej station was still observed in almost all cases for all measured parameters (Figure 8). As observed with the previous sampling campaigns, the Nordsøvej station had approximately 2-3 times higher average values of ATP, TDC, R2A, and HPC at 22 °C.

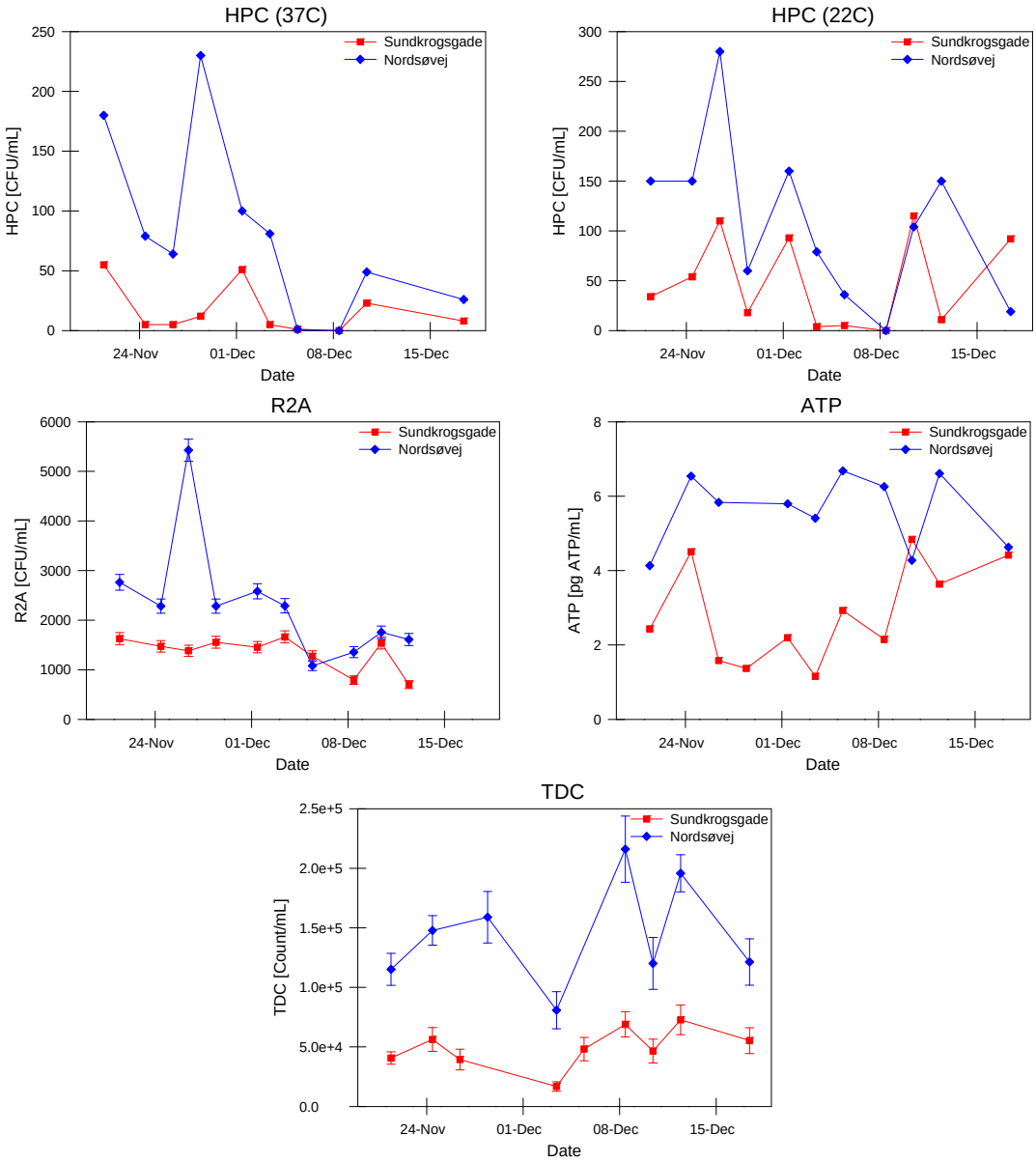


Figure 8: Samples collected at 10:00 at Nordsøvej and Sundkrogsgade for HPC (37 °C), HPC (22 °C), R2A, ATP, and TDC.

3.2.4 Comparing microbial water quality with panel results

In general, there was no relationship between the parameters monitored with the panels at the two stations and the microbial water quality analysis. One parameter that is known to affect microbial water quality is the

temperature. Microbial growth generally decreases with temperature. Another parameter that could show microbial growth is the DO. Since the drinking water is aerobic, changes in DO could be due to increased microbial activity, although decreased temperatures can also decrease the DO if the water is close to oxygen saturation.

At the Sundkrogsgade station there is an approximate 2 °C drop in temperature over the sampling campaign that seems to correspond with an increase in DO and an overall slight decrease in the R2A numbers (Figure 9). There was also a greater variation in DO and EC at the Sundkrogsgade station, although this did not fit any observed pattern of changes in the microbial water quality.

At the Nordsøvej station, the daily variation in temperature did not correspond to any observed pattern of increased microbial growth. The overall increased temperature and residence time in the building at the Nordsøvej station, could be causing an increase in microbial growth though, with temperatures that are approximately 4-7 °C higher than the water temperature at the Sundkrogsgade station.

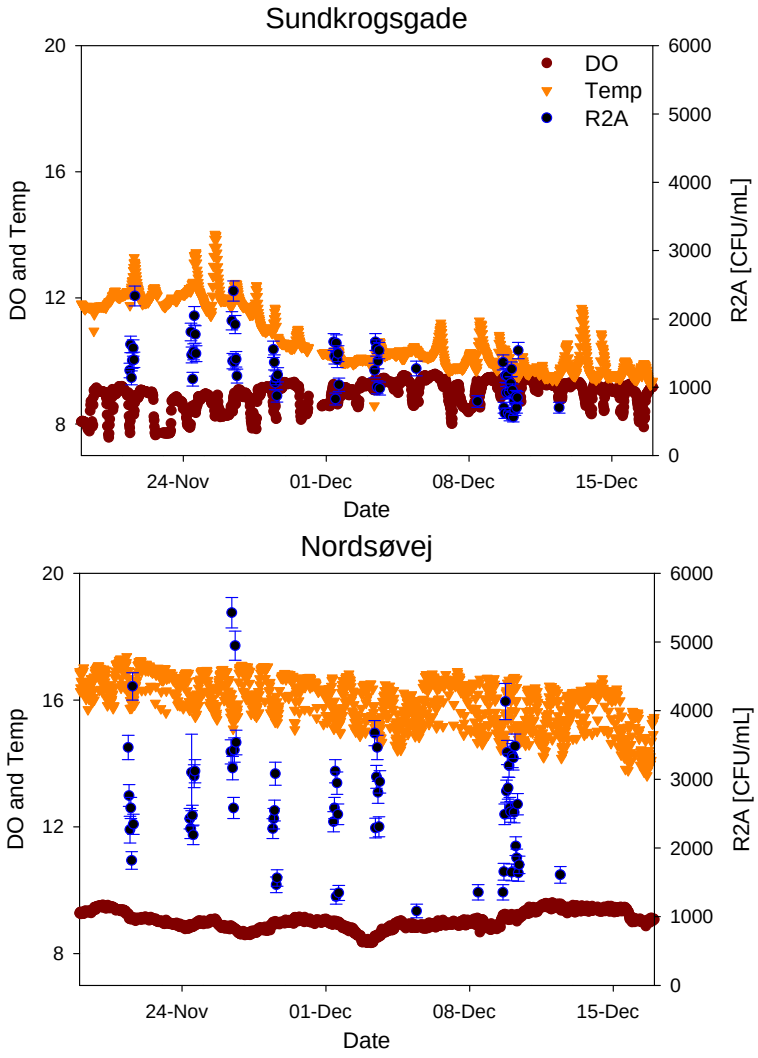


Figure 9: DO, Temperature, and R2A results at both monitoring stations during the sampling campaign.

3.3 Summary of microbial water quality

The general trend in the microbial water quality analysis was that higher counts and more variation was observed at the Nordsøvej station compared to the one at Sundkrogsgade. In general, the average HPC at 22

°C, ATP, and HPC using R2A agar were 2 to 3 times higher at the Nordsøvej station. The Nordsøvej station had approximately 5 times higher HPC at 37 °C, except for the overnight sampling campaign which saw 7 times more at Nordsøvej. All of these measured parameters point to lower microbial water quality at the Nordsøvej station, which is expected due to the increased water age, residence time, and increased temperature.

3.4 Zebra results

The Zebra analysers were set up at both stations and offer the benefit of giving a real time analysis of both the total particle count and the microbial count. The Zebra analysers have the benefit of giving data in real time and can give near continuous results because of the high sampling frequency. The Zebra results, showing the microbial count for both stations over the duration of the sampling campaign, is shown in Figure 10. It should be noted that the Zebra analyser at the Sundkrogsgade station was temporarily out of service at the beginning of the sampling campaign. Therefore the first Zebra results during the sampling campaign at Sundkrogsgade did not start until the beginning of December.

The microbial count from the Zebra analyser at the Nordsøvej station is generally the same or lower than at Sundkrogsgade (Figure 10). This is unexpected because, as discussed earlier, all of the other microbial water quality parameters were higher at the Nordsøvej station. In particular, the TDC results were almost always larger than the Zebra results at both stations (Figure 11). The Sundkrogsgade TDC counts are approximately 2 times larger than the Zebra results and the Nordsøvej Zebra results are around an order of magnitude lower than the TDC numbers (Figure 11).

Although the actual numbers might not directly correlate, it is also important to look at the general trends in the microbial water quality. In general most of the parameters seem to at least occasionally follow the same trends, although there is also deviations from these trends (Figure 11). At the Sundkrogsgade station, it is also difficult to access the trends due to the relatively short measuring period and with a period of missing data, although the HPC (37), R2A and Zebra results seem to correlate well with the limited data. The HPC (37C), ATP, and Zebra results at Nordhavn also seem to correlate relatively well. One of the important aspects of the Zebra would be to give an alarm if there was a spike in microbial counts. However, the microbial water quality varied very little, within a factor of 3 to 4, so no strong deterioration of the water quality was observed, and subsequently, no background for an alarm.

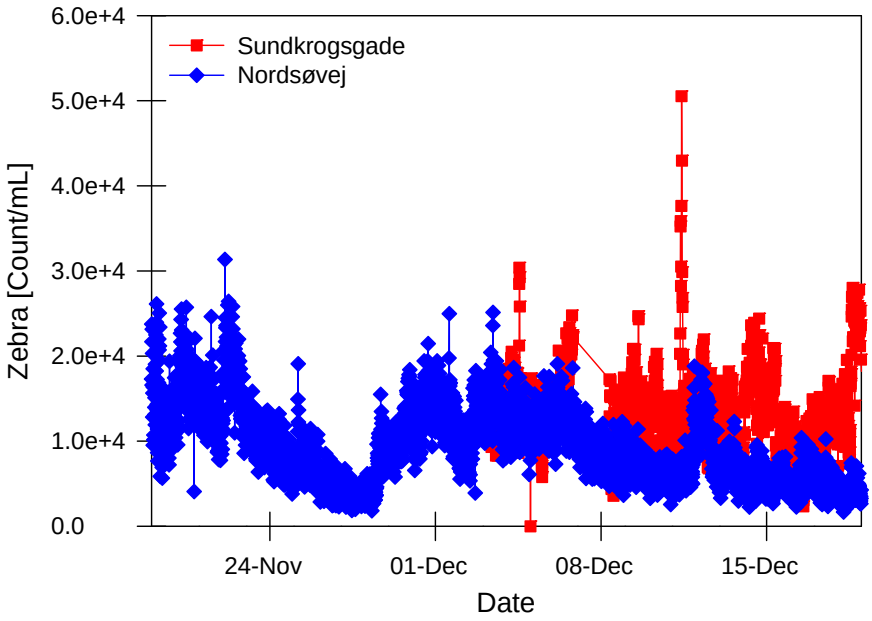
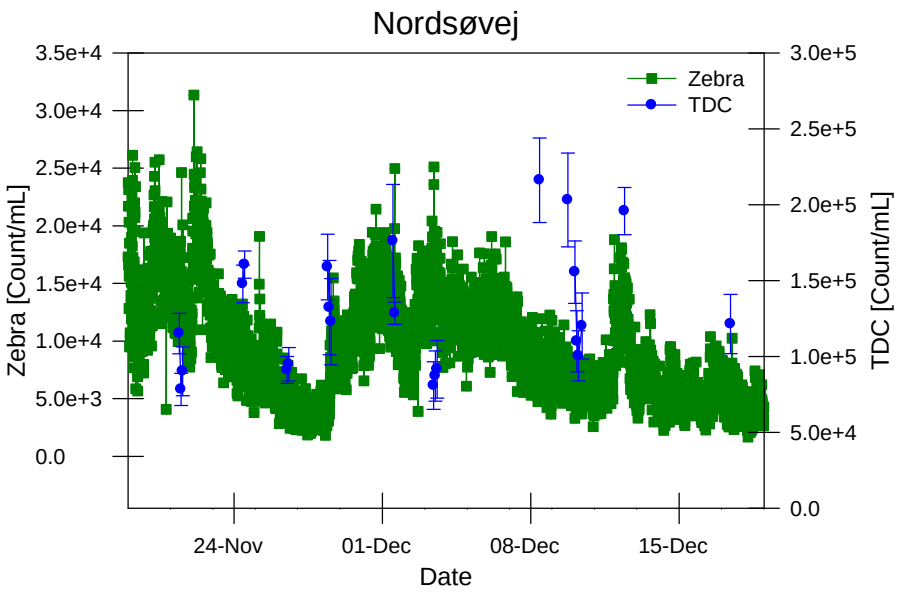
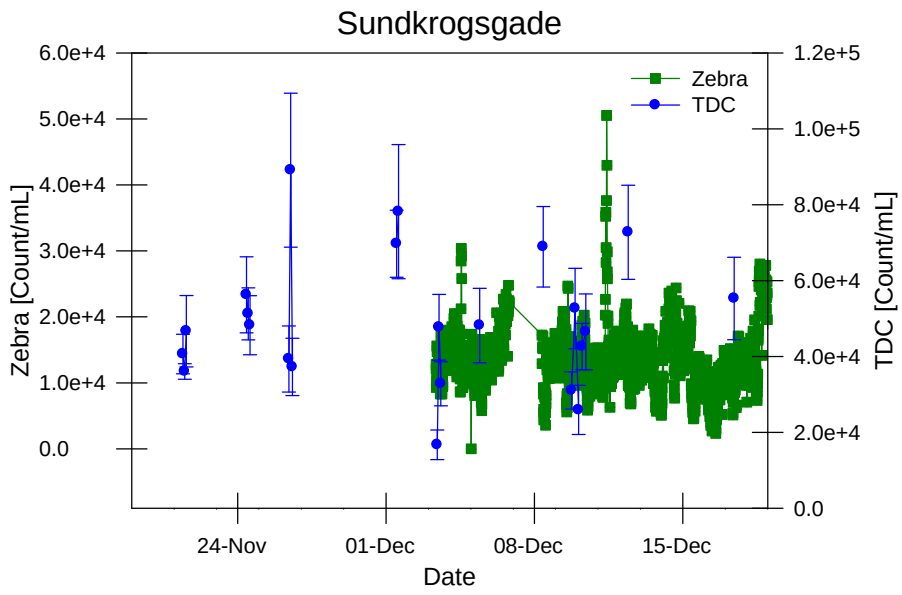
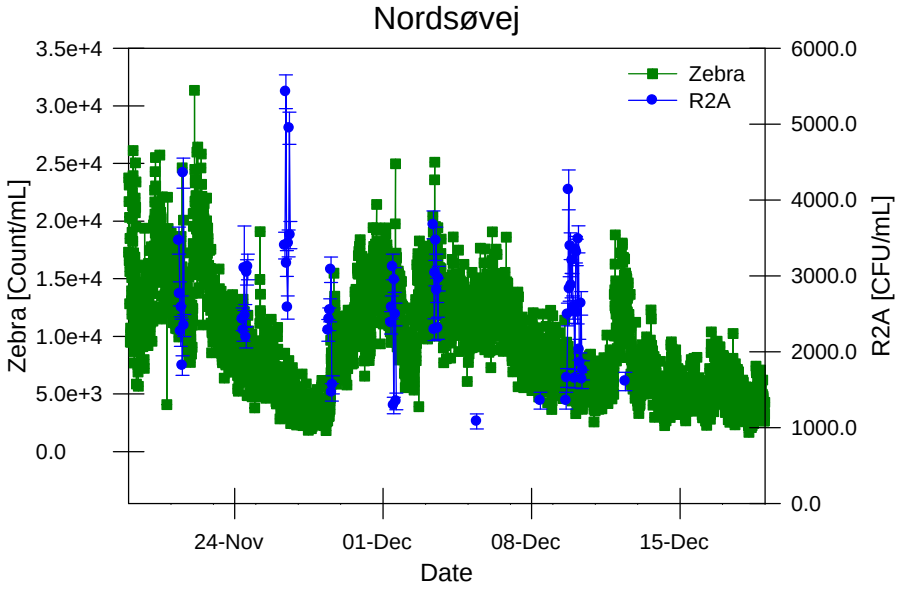
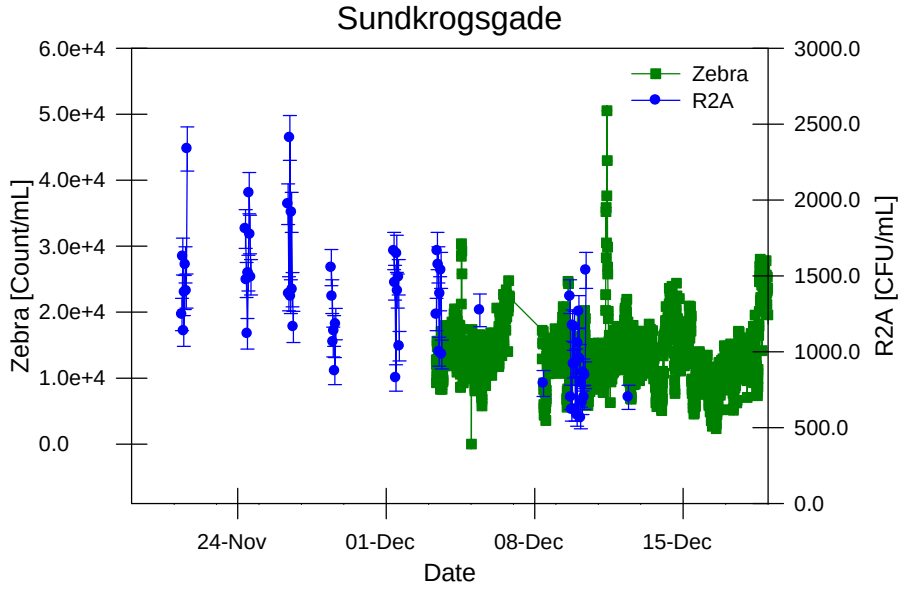


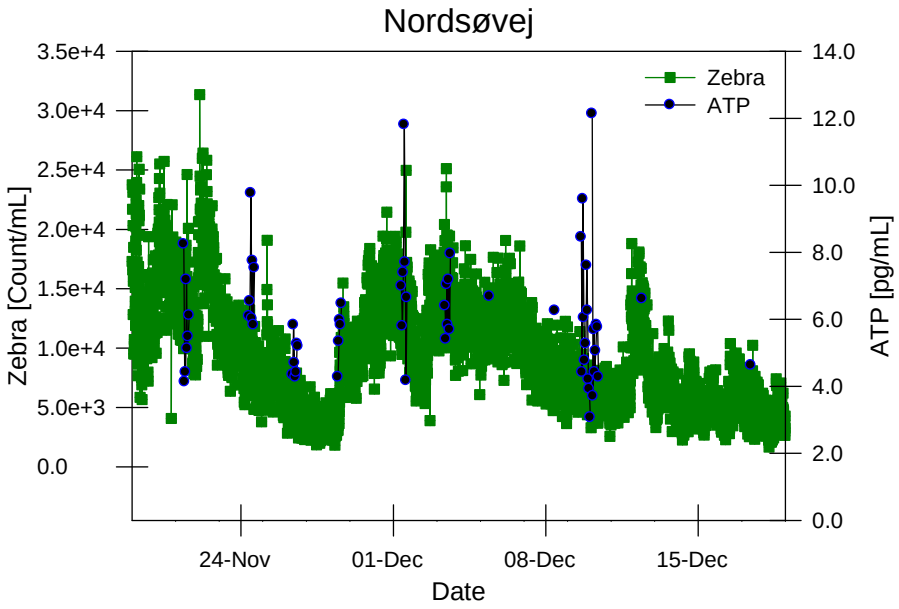
Figure 10: Zebra results showing the microbial count for both stations over the period of the sampling campaign.

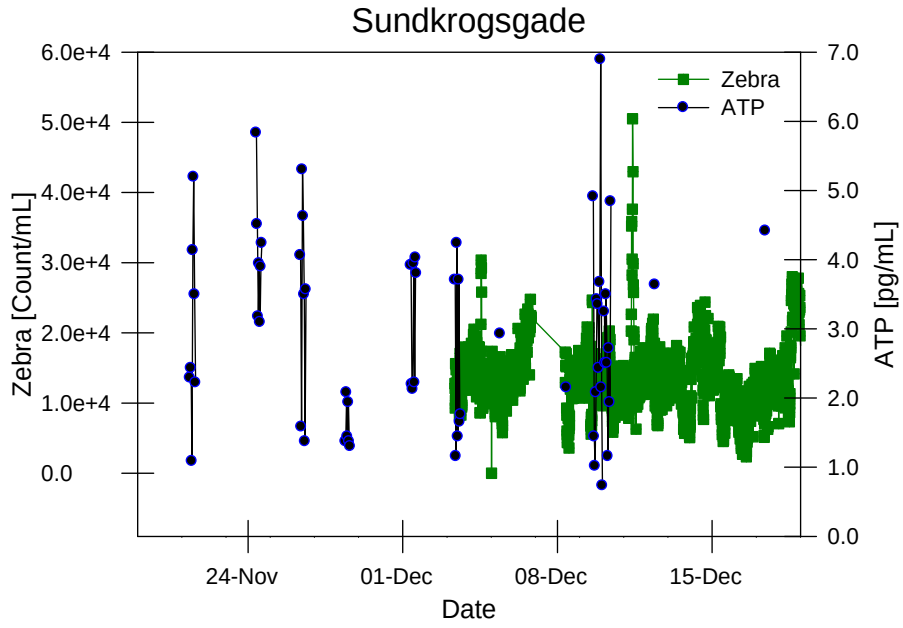

Nordsøvej
Zebra
TDC
Zebra [Count/mL]
TDC [Count/mL]
3.5e+4
3.0e+4
2.5e+4
2.0e+4
1.5e+4
1.0e+4
5.0e+3
0.0
3.0e+5
2.5e+5
2.0e+5
1.5e+5
1.0e+5
5.0e+4
0.0
24-Nov
01-Dec
08-Dec
15-Dec
Date

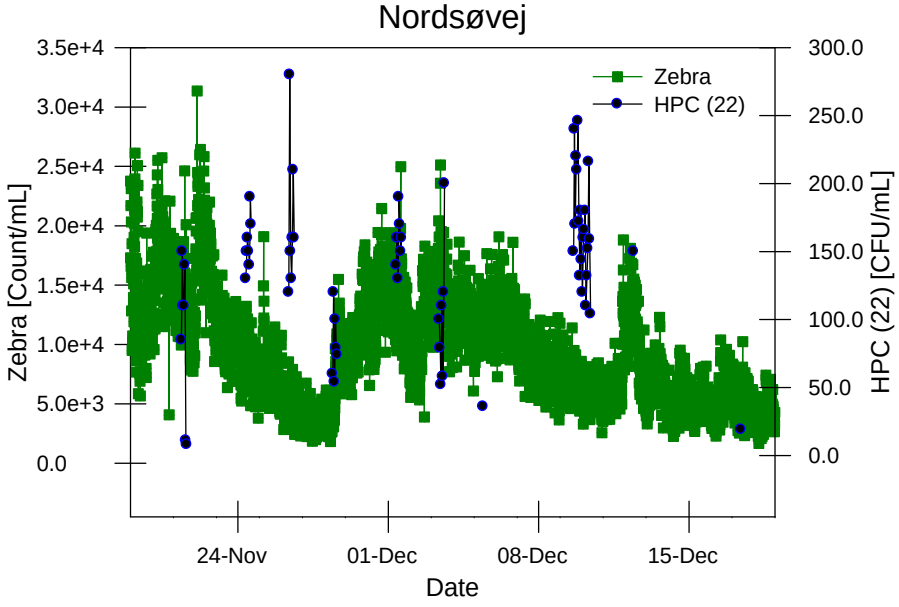

Sundkrogsgade
Zebra
TDC
Zebra [Count/mL]
TDC [Count/mL]
6.0e+4
5.0e+4
4.0e+4
3.0e+4
2.0e+4
1.0e+4
0.0
1.2e+5
1.0e+5
8.0e+4
6.0e+4
4.0e+4
2.0e+4
0.0
24-Nov
01-Dec
08-Dec
15-Dec
Date

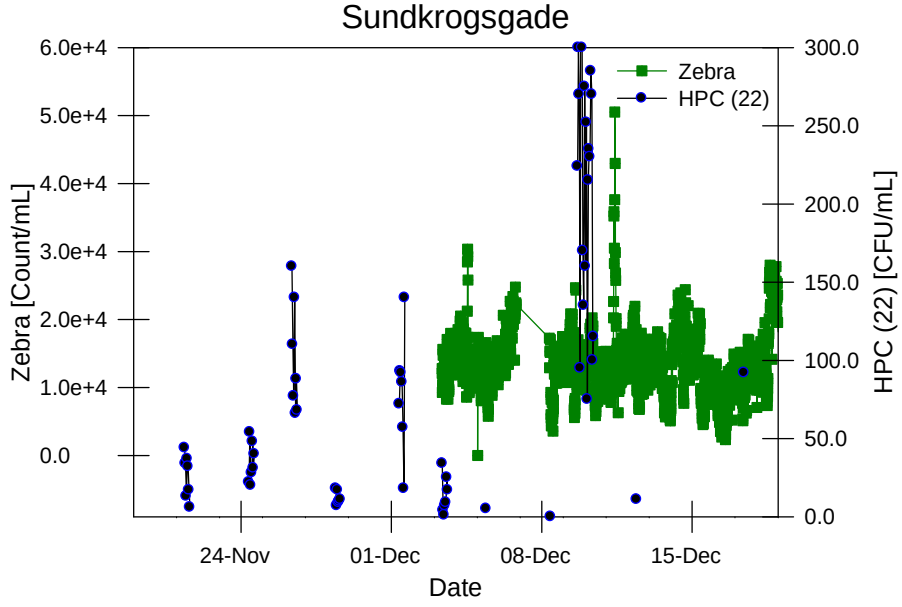

Nordsøvej
Zebra
R2A
Zebra [Count/mL]
R2A [CFU/mL]
3.5e+4
3.0e+4
2.5e+4
2.0e+4
1.5e+4
1.0e+4
5.0e+3
0.0
6000.0
5000.0
4000.0
3000.0
2000.0
1000.0
0.0
24-Nov
01-Dec
08-Dec
15-Dec
Date


Sundkrogsgade
Zebra
R2A
Zebra [Count/mL]
R2A [CFU/mL]
6.0e+4
5.0e+4
4.0e+4
3.0e+4
2.0e+4
1.0e+4
0.0
3000.0
2500.0
2000.0
1500.0
1000.0
500.0
0.0
24-Nov
01-Dec
08-Dec
15-Dec
Date


Nordsøvej
Zebra
ATP
Zebra [Count/mL]
ATP [pg/mL]
Date
24-Nov
01-Dec
08-Dec
15-Dec
3.5e+4
3.0e+4
2.5e+4
2.0e+4
1.5e+4
1.0e+4
5.0e+3
0.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0


Sundkrogsgade
Zebra
ATP
Zebra [Count/mL]
ATP [pg/mL]
Date
24-Nov
01-Dec
08-Dec
15-Dec
6.0e+4
5.0e+4
4.0e+4
3.0e+4
2.0e+4
1.0e+4
0.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0


Nordsøvej
Zebra
HPC (22)
Zebra [Count/mL]
HPC (22) [CFU/mL]
Date
24-Nov
01-Dec
08-Dec
15-Dec
3.5e+4
3.0e+4
2.5e+4
2.0e+4
1.5e+4
1.0e+4
5.0e+3
0.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0


Sundkrogsgade
Zebra
HPC (22)
Zebra [Count/mL]
HPC (22) [CFU/mL]
Date
24-Nov
01-Dec
08-Dec
15-Dec
6.0e+4
5.0e+4
4.0e+4
3.0e+4
2.0e+4
1.0e+4
0.0
300.0
250.0
200.0
150.0
100.0
50.0
0.0

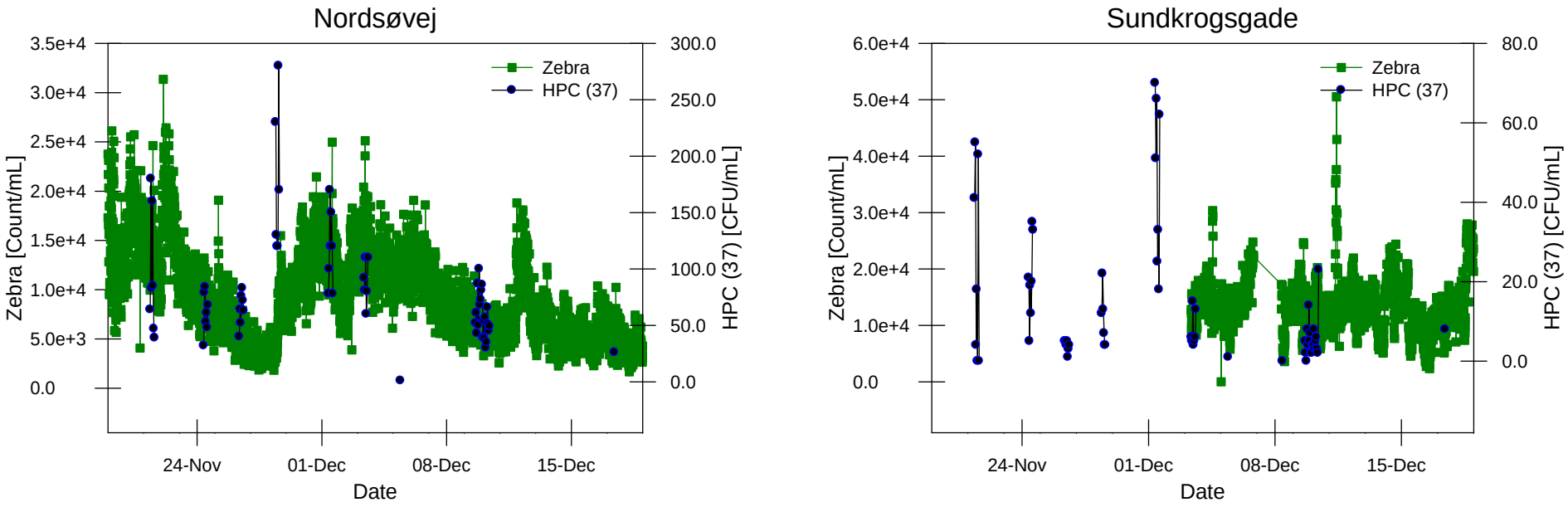


Figure 11: Zebra results at both Nordsøvej and Sundkrogsgade stations in combination with all TDC, R2A, ATP, HPC (22C), and HPC (37C) for the sampling period.

4. Conclusions

4.1 Monitoring panels:

- Parameters such as DO and EC tended to be more stable at the Nordsøvej station, whereas parameters such as temperature and Turbidity showed more rapid fluctuation at Nordsøvej.
- The temperature was higher at the Nordsøvej station (~14-17 °C) compared to the Sundkrogsgade station (~9-12 °C). This could be caused by the residence time in the building where the Nordsøvej station was located.
- In general, it was not possible to establish an obvious relationship between the parameters from the monitoring panels and the microbial water quality. Temperature and DO could be an indicator of microbial water quality, although during this study, there was only minimal evidence of this at the Sundkrogsgade station. At the Nordsøvej station, there was no clear pattern of changes in microbial water quality with the daily variations of temperature, although the overall increase in temperature at the Nordsøvej station could be causing the observed increased microbial growth.

4.2 Microbial water quality

All parameters used to analyse the microbial water quality were higher at the Nordsøvej station. The average ATP, HPC at 22 °C, R2A, and TDC results were 2 to 3 times larger at the Nordsøvej station. Also, these parameters had a larger variation at Nordsøvej than Sundkrogsgade. This was expected though as the Nordsøvej station had a higher temperature, higher water age, and longer residency time in the distribution system allowing for increased microbial growth. The one notable exception was for the HPC at 22 °C during the extended overnight sampling campaign. During this period, Sundkrogsgade had higher counts than Nordsøvej for a majority of the samples and showed a larger variation over the sampling period. This could have been due to the long sampling times and problems with preserving the samples at the Sundkrogsgade station during this sampling event.

Although the monitoring panel at Sundkrogsgade showed some variation in the water composition due to shifting water sources, the microbial water quality was relatively stable. The samples collected at 10:00 showed some variation over the sampling period for the HPC using yeast extract at 22 °C and 37 °C. Despite the variation, the general trends of higher counts and greater variation at the Nordsøvej station was still observed in almost all cases for all measured parameters.

4.3 Zebra results

The Zebra results were surprising because the Nordsøvej station had similar or lower Zebra counts than the Sundkrogsgade station. When comparing the TDC to the Zebra counts, the biological counts for the Zebra were around 2 times lower than the TDC at the Sundkrogsgade station, and an order of magnitude lower than the TDC values although at the Nordsøvej station. This may not be as important as in capturing the trends of the microbial water quality.

Both stations seemed to have a good correlation to the HPC (37C) results and the Nordsøvej station zebra results also correlated well with the ATP. The limited Zebra data made it difficult to assess most of the measured parameters at the Sundkrogsgade station, although the R2A values also seemed to correlate well with the Zebra results at Sundkrogsgade.

5. Appendix

Table 1: All measured microbial water quality results for Sundkrogsgade station.

	R2A		HPC		ATP		TDC		
Date and time	R2A [CFU/ml]	E.o.m.	HPC 22 °C [CFU/mL]	HPC 37 °C [CFU/mL]	ATP [pg ATP/ml]	Std. Dev	TDC [Count/mL]	Std. Dev	COV
21-11-14 09:00	1.25E+03	1.06E+02	44	41	2.3	-			
21-11-14 10:00	1.63E+03	1.22E+02	34	55	2.4	-	4.06E+04	5.20E+03	13%
21-11-14 11:00	1.14E+03	1.02E+02	13	4	1.1	-			
21-11-14 12:00	1.39E+03	1.12E+02	37	18	4.1	-	3.60E+04	2.05E+03	6%
21-11-14 13:00	1.57E+03	1.20E+02	32	-	5.2	-			
21-11-14 14:00	1.40E+03	1.13E+02	17	52	3.5	-	4.67E+04	9.41E+03	20%
21-11-14 15:00	2.34E+03	1.46E+02	6	<1	2.2	-			
24-11-14 09:00	1.81E+03	1.28E+02	22	21	5.8	0.8			
24-11-14 10:00	1.47E+03	1.16E+02	54	5	4.5	1.2	5.62E+04	1.00E+04	18%
24-11-14 11:00	1.12E+03	1.01E+02	20	19	3.2	0.6			
24-11-14 12:00	1.52E+03	1.17E+02	28	12	3.9	1.0	5.13E+04	6.86E+03	13%
24-11-14 13:00	2.05E+03	1.36E+02	48	20	3.1	1.0			
24-11-14 14:00	1.77E+03	1.27E+02	31	35	3.9	1.1	4.82E+04	7.77E+03	16%
24-11-14 15:00	1.49E+03	1.16E+02	40	33	4	0.7			
26-11-14 09:00	1.97E+03	1.34E+02	160	5	4	-			
26-11-14 10:00	1.38E+03	1.12E+02	110	5	2	-	3.9E+04	8.7E+03	22%
26-11-14 11:00	2.41E+03	1.48E+02	77	4	5	1.8			
26-11-14 12:00	1.36E+03	1.11E+02	140	5	5	-	8.9E+04	2.0E+04	23%
26-11-14 13:00	1.92E+03	1.32E+02	66	1	3	-			
26-11-14 14:00	1.41E+03	1.13E+02	88	3	1	-	3.72E+04	7.55E+03	20%
26-11-14 15:00	1.16E+03	1.03E+02	68	4	4	-			
28-11-14 10:00	1.55E+03	1.19E+02	18	12	1	-	2.07E+04	1.32E+04	64%
28-11-14 11:00	1.36E+03	1.11E+02	7	22	2	-			

	R2A		HPC		ATP		TDC		
Date and time	R2A [CFU/ml]	E.o.m.	HPC 22 °C [CFU/mL]	HPC 37 °C [CFU/mL]	ATP [pg ATP/ml]	Std. Dev	TDC [Count/mL]	Std. Dev	COV
28-11-14 12:00	1.06E+03	9.83E+01	17	13	1	-	3.13E+04	1.20E+04	38%
28-11-14 13:00	1.14E+03	1.02E+02	9	7	2	-			
28-11-14 14:00	8.73E+02	8.91E+01	10	4	1	-	4.32E+04	2.22E+04	51%
28-11-14 15:00	1.18E+03	1.04E+02	11	4	1	-			
01-12-14 09:00	1.66E+03	1.23E+02	72	70	4	0.6			
01-12-14 10:00	1.45E+03	1.15E+02	93	51	2	0.9	5.93E+04	2.39E+04	40%
01-12-14 11:00	8.27E+02	8.67E+01	92	66	2	1.3			
01-12-14 12:00	1.65E+03	1.22E+02	86	25	4	1.5	6.97E+04	8.84E+03	13%
01-12-14 13:00	1.40E+03	1.13E+02	57	33	2	1.2			
01-12-14 14:00	1.49E+03	1.16E+02	18	18	4	0.8	7.8E+04	1.8E+04	23%
01-12-14 15:00	1.04E+03	9.71E+01	140	62	4	1.1			
03-12-14 09:00	1.25E+03	1.06E+02	34	6	4	-			
03-12-14 10:00	1.66E+03	1.23E+02	4	5	1	-	1.7E+04	3.9E+03	23%
03-12-14 11:00	1.57E+03	1.20E+02	1	15	4	0.8			
03-12-14 12:00	1.00E+03	9.53E+01	7	4	1	-	4.77E+04	8.66E+03	18%
03-12-14 13:00	1.38E+03	1.12E+02	9	5	4	-			
03-12-14 14:00	1.54E+03	1.18E+02	25	6	2	-	3.28E+04	5.79E+03	18%
03-12-14 15:00	9.82E+02	9.45E+01	17	13	2	0.6			
05-12-14 10:00	1.27E+03	1.08E+02	5	1	3	-	4.82E+04	9.80E+03	20%
08-12-14 10:00	7.91E+02	8.48E+01	<1	<1	2	-	6.89E+04	1.06E+04	15%
09-12-14 15:00	3.83E+03	1.87E+02	895	48	5	-			
09-12-14 16:00	1.36E+03	1.11E+02	224	5	1	-			
09-12-14 17:00	7.00E+02	7.98E+01	300	2	1	-			
09-12-14 18:00	6.18E+02	7.50E+01	270	-	2	-	3.11E+04	4.89E+03	16%
09-12-14 19:00	1.17E+03	1.03E+02	95	8	3	-			
09-12-14 20:00	9.18E+02	9.14E+01	500	4	3	-			
09-12-14 21:00	1.16E+03	1.03E+02	300	14	2	-			
09-12-14 22:00	6.18E+02	7.50E+01	170	5	4	0.7	5.26E+04	1.06E+04	20%
09-12-14 23:00	9.27E+02	9.18E+01	135	7	7	5.2			

	R2A		HPC		ATP		TDC		
Date and time	R2A [CFU/ml]	E.o.m.	HPC 22 °C [CFU/mL]	HPC 37 °C [CFU/mL]	ATP [pg ATP/ml]	Std. Dev	TDC [Count/mL]	Std. Dev	COV
10-12-14 00:00	5.82E+02	7.27E+01	275	2	2	-			
10-12-14 01:00	1.05E+03	9.79E+01	160	2	1	-			
10-12-14 02:00	1.26E+03	1.07E+02	252	4	2	-	2.59E+04	6.48E+03	25%
10-12-14 03:00	9.55E+02	9.32E+01	75	4	3	0.8			
10-12-14 04:00	5.64E+02	7.16E+01	215	8	3	-			
10-12-14 05:00	7.91E+02	8.48E+01	235	3	3	0.7			
10-12-14 06:00	6.64E+02	7.77E+01	230	5	3	-	4.26E+04	6.09E+03	14%
10-12-14 07:00	8.64E+02	8.86E+01	285	6	1	-			
10-12-14 08:00	7.00E+02	7.98E+01	270	3	3	-			
10-12-14 09:00	8.45E+02	8.77E+01	100	2	2	-			
10-12-14 10:00	1.54E+03	1.18E+02	115	23	5	-	4.65E+04	9.99E+03	21%
12-12-14 10:00	7.00E+02	7.98E+01	11	>300	4	-	7.27E+04	1.24E+04	17%
17-12-14 10:00			92	8	4	-	5.53E+04	1.09E+04	20%

Table 2: All measured microbial water quality results for Nordsøvej station.

R2A			HPC		ATP		TDC		
Date and time	R2A [CFU/ml]	E.o.m.	HPC 22 °C [CFU/mL]	HPC 37 °C [CFU/mL]	ATP [pg ATP/ml]	Std. Dev	TDC [Count/mL]	Std. Dev	COV
21-11-14 09:00	3.5E+03	1.8E+02	85	64	8.2	-			
21-11-14 10:00	2.8E+03	1.6E+02	150	180	4.1	-	1.2E+05	1.3E+04	12%
21-11-14 11:00	2.3E+03	1.9E+02	110	83	4.4	-			
21-11-14 12:00	2.6E+03	1.5E+02	110	160	7.2	-	7.8E+04	1.1E+04	14%
21-11-14 13:00	1.8E+03	1.3E+02	140	85	5.1	-			
21-11-14 14:00	4.4E+03	2.0E+02	11	47	5.5	-	9.0E+04	1.6E+04	18%
21-11-14 15:00	2.3E+03	1.5E+02	8	39	6.1	-			
24-11-14 09:00	2.4E+03	1.5E+02	130	32	6.1	1.5			
24-11-14 10:00	2.3E+03	1.4E+02	150	79	6.5	1.7	1.5E+05	1.2E+04	8%
24-11-14 11:00	3.1E+03	5.6E+02	160	84	9.8	1.0			
24-11-14 12:00	2.5E+03	1.5E+02	150	53	6.0	1.0	1.6E+05	9.0E+03	6%
24-11-14 13:00	2.2E+03	1.4E+02	140	61	7.7	1.1			
24-11-14 14:00	3.0E+03	1.7E+02	190	48	5.8	1.6	1.2E+05	4.3E+04	36%
24-11-14 15:00	3.1E+03	1.7E+02	170	68	8	0.8			
26-11-14 09:00	3.4E+03	1.8E+02	120	40	4	-			
26-11-14 10:00	5.4E+03	2.2E+02	280	64	6	-	1.0E+05	4.1E+04	41%
26-11-14 11:00	3.2E+03	1.7E+02	150	52	5	-			
26-11-14 12:00	2.6E+03	1.5E+02	130	76	4	-	9.1E+04	8.8E+03	10%
26-11-14 13:00	3.4E+03	1.8E+02	160	83	4	-			
26-11-14 14:00	4.9E+03	2.1E+02	210	72	5	-	9.5E+04	1.1E+04	12%
26-11-14 15:00	3.5E+03	1.8E+02	160	63	5	-			
28-11-14 10:00	2.3E+03	1.4E+02	60	230			1.6E+05	2.2E+04	14%
28-11-14 11:00	2.4E+03	1.5E+02	120	130	4	-			
28-11-14 12:00	2.5E+03	1.5E+02	54	120	5	-	1.3E+05	3.1E+04	24%
28-11-14 13:00	3.1E+03	1.7E+02	100	120	6	-			
28-11-14 14:00	1.5E+03	1.2E+02	79	280	6	-	1.2E+05	2.8E+04	23%
28-11-14 15:00	1.6E+03	1.2E+02	74	170	6	-			

R2A			HPC		ATP		TDC		
Date and time	R2A [CFU/ml]	E.o.m.	HPC 22 °C [CFU/mL]	HPC 37 °C [CFU/mL]	ATP [pg ATP/ml]	Std. Dev	TDC [Count/mL]	Std. Dev	COV
01-12-14 09:00	2.4E+03	1.5E+02	140	78	7	1.5			
01-12-14 10:00	2.6E+03	1.5E+02	160	100	6	0.7	1.4E+05	5.6E+04	40%
01-12-14 11:00	3.1E+03	1.7E+02	130	170	7	0.9			
01-12-14 12:00	1.3E+03	1.1E+02	190	120	12	1.8	1.8E+05	3.7E+04	21%
01-12-14 13:00	2.9E+03	1.6E+02	170	150	8	1.7			
01-12-14 14:00	2.5E+03	1.5E+02	150	120	4	0.7	1.3E+05	7.3E+03	6%
01-12-14 15:00	1.3E+03	1.1E+02	160	78	7	0.9			
03-12-14 09:00	3.7E+03	1.8E+02	100	92	6	-			
03-12-14 10:00	2.3E+03	1.4E+02	79	81	5	-	8.1E+04	1.6E+04	19%
03-12-14 11:00	3.0E+03	1.7E+02	52	110	7	-			
03-12-14 12:00	3.5E+03	1.8E+02	110	60	6	-	8.7E+04	1.7E+04	19%
03-12-14 13:00	2.8E+03	1.6E+02	58	80	7	-			
03-12-14 14:00	2.3E+03	1.4E+02	120	110	6	-	9.2E+04	1.9E+04	21%
03-12-14 15:00	3.0E+03	1.6E+02	200	110	8	-			
05-12-14 10:00	1.1E+03	9.9E+01	36	1	7	-	9.1E+04	4.3E+04	48%
08-12-14 10:00	1.4E+03	1.1E+02	<1	<1	6	0.8	2.2E+05	2.8E+04	13%
09-12-14 15:00	1.4E+03	1.1E+02	150	52	8	-			
09-12-14 16:00	1.7E+03	1.2E+02	240	61	4	-			
09-12-14 17:00	2.5E+03	1.5E+02	170	43	10	-			
09-12-14 18:00	4.1E+03	2.6E+02	220	87	6	-	2.0E+05	3.1E+04	15%
09-12-14 19:00	2.8E+03	1.6E+02	210	50	5	-			
09-12-14 20:00	3.4E+03	1.8E+02	246	100	5	-			
09-12-14 21:00	2.9E+03	1.6E+02	172	68	8	-			
09-12-14 22:00	3.2E+03	1.7E+02	132	73	6	-	1.3E+05	4.4E+04	35%
09-12-14 23:00	2.6E+03	1.5E+02	180	81	4	-			
10-12-14 00:00	2.5E+03	1.5E+02	144	86	4	-			
10-12-14 01:00	1.6E+03	1.2E+02	120	39	3	-			

R2A			HPC		ATP		TDC		
Date and time	**R2A [CFU/ml]**	**E.o.m.**	**HPC 22 °C [CFU/mL]**	**HPC 37 °C [CFU/mL]**	**ATP [pg ATP/ml]**	**Std. Dev**	**TDC [Count/mL]**	**Std. Dev**	**COV**
10-12-14 02:00	3.3E+03	1.7E+02	160	54	25	5.1	1.6E+05	2.1E+04	13%
10-12-14 03:00	3.3E+03	1.7E+02	166	38	12	-			
10-12-14 04:00	2.5E+03	1.5E+02	180	57	4	-	1.1E+05	2.0E+04	18%
10-12-14 05:00	3.5E+03	1.8E+02	110	30	6	-			
10-12-14 06:00	2.0E+03	1.4E+02	132	35	4	-	1.0E+05	1.7E+04	16%
10-12-14 07:00	1.9E+03	1.3E+02	152	66	5	-			
10-12-14 08:00	2.6E+03	1.5E+02	216	51	6	-			
10-12-14 09:00	1.6E+03	1.2E+02	159	45	6	-			
10-12-14 10:00	1.8E+03	1.3E+02	104	49	4	-	1.2E+05	2.2E+04	18%
12-12-14 10:00	1.6E+03	1.2E+02	150	>300	7	-	2.0E+05	1.6E+04	8%
17-12-14 10:00	-	-	19	26	5	-	1.2E+05	1.9E+04	16%

Table 3: Summarized table of average values for all measured microbial water quality parameters at Nordsøvej and Sundkrogsgade stations.

	Summary Nordsøvej					Summary Sundkrogsgade				
	HPC 22 °C [CFU/mL]	HPC 37 °C [CFU/mL]	R2A [CFU/mL]	ATP [pg ATP/mL]	TDC [count/mL]	HPC 22 °C [CFU/mL]	HPC 37 °C [CFU/mL]	R2A [CFU/mL]	ATP [pg ATP/mL]	TDC [count/mL]
Average (9-15)	127.3	98.6	2.8E+03	6.2	1.2E+05	45.4	20.1	1.5E+03	3.0	4.9E+04
std dev 9-15	53.7	52.2	8.5E+02	1.6	3.2E+04	40.9	19.9	3.6E+02	1.3	1.9E+04
Average (all)	137.3	83.1	2.7E+03	6.4	1.3E+05	110.5	15.8	1.3E+03	3.0	4.9E+04
std dev (all)	55.6	48.9	8.6E+02	2.9	4.1E+04	143.2	18.0	5.2E+02	1.3	1.7E+04
Average all (excluding overnight)	123.3	94.6	2.7E+03	6.2	1.3E+05	44.8	19.3	1.4E+03	3.0	5.2E+04
std dev all (excluding overnight)	56.1	54.2	9.0E+02	1.5	4.1E+04	40.9	19.7	3.7E+02	1.3	1.8E+04
Average 10 am	118.8	90.0	2.3E+03	5.6	1.4E+05	53.6	18.3	1.3E+03	2.8	4.9E+04
std dev 10 am	76.5	72.8	1.2E+03	1.0	4.5E+04	45.1	20.7	3.4E+02	1.3	1.7E+04
Average overnight (15-10)	168.2	58.3	2.6E+03	6.8	1.4E+05	255.1	8.2	1.1E+03	2.8	4.0E+04
std dev overnight (15-10)	41.0	19.2	7.7E+02	4.8	4.2E+04	179.4	10.9	7.1E+02	1.5	1.1E+04
Averages over 9-15										
Average 9:00	115.0	61.2	3.1E+03	6.4		66.4	28.6	1.6E+03	4.0	
Std dev 9:00	22.4	25.2	6.2E+02	1.4		55.5	27.4	3.3E+02	1.3	
Average 10:00	146.5	122.3	2.9E+03	5.5	1.3E+05	52.2	22.2	1.5E+03	2.2	3.8E+04

	Summary Nordsøvej					Summary Sundkrogsgade				
	HPC 22 °C [CFU/mL]	**HPC 37 °C [CFU/mL]**	**R2A [CFU/mL]**	**ATP [pg ATP/mL]**	**TDC [count/mL]**	**HPC 22 °C [CFU/mL]**	**HPC 37 °C [CFU/mL]**	**R2A [CFU/mL]**	**ATP [pg ATP/mL]**	**TDC [count/mL]**
Std dev 10:00	77.5	66.9	1.2E+03	0.9	3.5E+04	42.0	24.1	1.1E+02	1.2	1.6E+04
Average 11:00	120.3	104.8	2.9E+03	6.3		35.0	21.7	1.4E+03	3.0	
Std dev 11:00	38.3	41.5	4.0E+02	2.2		39.1	23.0	5.5E+02	1.6	
Average 12:00	124.0	98.2	2.5E+03	6.7	1.2E+05	52.5	12.8	1.3E+03	3.3	5.9E+04
Std dev 12:00	45.5	41.9	6.9E+02	2.7	4.1E+04	50.9	7.9	2.5E+02	1.4	2.1E+04
Average 13:00	128.0	96.5	2.7E+03	6.4		36.8	13.2	1.6E+03	3.3	
Std dev 13:00	41.9	32.4	6.0E+02	1.4		24.3	13.2	3.5E+02	1.2	
Average 14:00	126.7	112.8	3.1E+03	5.4	1.1E+05	31.5	19.7	1.4E+03	2.6	4.9E+04
Std dev 14:00	73.8	87.4	1.3E+03	0.6	1.9E+04	28.6	20.0	3.0E+02	1.3	1.8E+04
Average 15:00	128.7	88.0	2.5E+03	6.7		47.0	23.2	1.4E+03	2.8	
Std dev 15:00	72.5	46.3	8.8E+02	1.0		51.0	24.7	5.1E+02	1.2	

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